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PORTON TECHNICAL PAPER No. 967

THE INFLUENCE OF PLASMA LEVELS OF P2S (AND ADJUVANT TREATMENT WITH ATROPINE) UPON SURVIVAL OF RABBITS AND DOGS POISONED WITH GB OR VX. [S]

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BY

D.R.DAVIES AND R.P.BRADSHAW, Lt.Col. R.A.M.C.

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DATE: April 25th, 1967

(7) [THE INFLUENCE OF PLASMA LEVELS OF P2S (AND ADJUVANT TREATMENT WITH ATROPINE) UPON SURVIVAL OF RABBITS AND DOGS POISONED WITH GB OR VX] [S]. (8)

~~By~~

(10) D.R. Davies [redacted] R.P. Bradshaw, ~~Lt. Col. R.A.M.C.~~

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(14) PTP-967

SUMMARY

The importance of the concentration of P2S in the plasma of rabbits and dogs has been studied as a determinant of death or survival after poisoning by GB or VX and treatment post poisoning with atropine. It has been shown that 4 ~~µg~~ P2S/ml of plasma is a critical concentration for survival against 10 x LD50 of GB or VX provided that atropine is given immediately after poisoning or on the objective signs of poisoning.

micrograms

(Sgd.) F.W. Beswick,
Superintendent,
Medical Research Division.

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mlh (080 450)

(Sgd.) W.S.S. Ladell,
Assistant Director,
Medical.

DRD/RPB/JCC

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PORTON TECHNICAL PAPER NO. 967
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THE INFLUENCE OF PLASMA LEVELS OF P2S (AND ADJUVANT TREATMENT WITH ATROPINE) UPON SURVIVAL OF RABBITS AND DOGS POISONED WITH GB OR VX

By

D.R. Davies and R.P. Bradshaw, Lt. Col.R.A.M.C.

INTRODUCTION

One approach to the treatment of nerve agent poisoning in the field is to give oxime orally, prior to poisoning and atropine by self injection immediately after poisoning. The effective application of this approach is dependent upon (a) the development of a suitable formulation of oxime which when given orally, will produce an adequate plasma level over a long period, with a minimum number of doses and long intervals between them and (b) the ability of the soldier to administer atropine to himself at the optimum time.

Just what constitutes an "adequate" level of oxime in the plasma, has not been clearly defined, although American workers (1) have concluded "that the blood oxime level at the start of an exposure appears to be a determinant of eventual death or survival after exposure to sarin (GB) and subsequent treatment with a standard dose of atropine". These same workers also stated that "a blood level of oxime of 3 μg/ml is suggested for estimating in man the optimal dose and dosing schedule for oral employment of quaternary - 2-formylpyridine oxime to antagonise the toxic effects of inhaled phosphorus anticholinesterases". Despite the firmness of this

recommendation it is difficult to assess its true value since several animals in their series died with oxime levels much greater than 3 μg/ml and survival could not be forecast purely upon the basis of the level of oxime in the plasma. Indeed the mean oxime levels of the survivors namely 6.76 μg/ml, was not significantly different from those that died, 5.03 μg/ml. Furthermore the challenge given to different animals varied.

Although many studies have been carried out to determine the dose of oxime (in conjunction with a standard dose of atropine) necessary to protect animals against large doses of nerve agent (2), (3), in none of them have plasma oxime levels been concurrently determined. Since individual variation in the blood level, following a standard dose of oxime, is so wide, both in animals and man, experiments of this kind are at the best of limited value in the present context. It has therefore been necessary to determine the critical plasma oxime level necessary for survival of animals predosed with P2S, challenged with isopropylmethylphosphonofluoridate (GB) or with O-ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate (VX), followed by intramuscular atropine given immediately after poisoning.

MATERIALS

Animals Rabbits of both sexes and of average weight of 2 kg were used.
Dogs were of mixed breed and sex.

Oxime 2Hydroxyiminomethyl-N-methylpyridiniummethylmethane sulphonate (P2S) was obtained from Processing Division and was 98-99% pure.

Agent Isopropylmethylphosphonofluoridate (GB) and O-ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate (VX) were produced by C.R.D.

METHODS

The Estimation of P2S. For rabbit blood, 0.1 ml of plasma was mixed with 0.1 ml barium hydroxide (0.15M) and 0.4 ml of water. 0.1 ml of zinc sulphate (0.15M) and one drop of diethyl ether (to prevent frothing) were added and the mixture was then centrifuged; 0.3 ml of the supernatant were added to 0.02 ml of 20% caustic soda in a 1 cm cell and the optical density was read at 335 mμ. The concentration of P2S was then read off from a suitably constructed calibration curve.

For dogs a larger sample of plasma was used (0.5 ml) and the volumes of the reagents scaled up accordingly. Otherwise the procedure was exactly the same.

ANIMAL EXPERIMENTS

Before the main experiments a limited number of studies were carried out upon the variation in plasma levels of P2S with time.

Subsequently the general scheme was similar in all cases, differing only in detail in individual experiments. A blood sample was taken before the administration of P2S and subsequently at varying intervals depending upon the experiment. After oxime was given a second sample was obtained. Immediately after this the nerve agent was given and, then very quickly, 17.4 mg/kg atropine sulphate, intramuscularly.

RESULTS

1. The variation in Plasma P2S levels in Rabbits and Dogs after intramuscular injection of oxime.

Rabbits. Two dose levels were investigated, 15 and 10 mg/kg. Three animals were examined at each dose. The results are shown in figures 1 and 2. At 15 mg/kg, the response of individual animals was reasonably consistent; the maximum observed values, of 10-14 µg/ml were reached in 3-6 minutes and levels of 4 µg/ml or greater were maintained for approximately 20 minutes. At 10 mg/kg the response was more variable, the maximum observed values were lower, 7-9 µg/ml and they occurred in 3-4 minutes. The rate at which the P2S level fell was however, less constant. In two animals the 4 µg/ml level or greater was maintained for only about 7 minutes. In the third animal it remained at this level for more than double the time (See Figs. 1 and 2).

Dogs. In dogs given 15 mg/kg i.m. the maximum observed value was similar to that for rabbits, but the 4 µg/ml or greater level was maintained for a longer period, viz at least 60 minutes. (Fig. 3).

2. The variation of P2S levels in the Plasma of Rabbits following Oral Administration.

Four animals were given 100 mg/kg of P2S orally in gelatine capsules. The variation in plasma levels is shown in Figure 4. Much greater individual variation was seen when the oxime was given by this route than when it was given intramuscularly. The maximum observed values varied from 2 µg/ml to approximately 8 µg/ml and the period during which the 4 µg/ml level was maintained varied in three animals from roughly 2 to 4 hours. In the fourth animal the 4 µg/ml level or greater was not reached and the peak value of 2 µg/ml occurred only after 2 hours.

3. Plasma levels of P2S and Survival of Rabbits given GB by different Routes as Influenced by the Route of Administration of Atropine.

Eight rabbits were given 5 x LD50 of GB intramuscularly, ten minutes after 30 mg/kg P2S, also intramuscularly. Atropine sulphate (17.4 mg/kg) was given by the same route, one minute after the GB. Seven animals survived. The plasma P2S levels varied in the eight rabbits at the time of challenge between 16-25 µg/ml. (See Figure 5).

These plasma levels were high, and experiments which were being carried out on humans relating oral dose to plasma level of P2S suggested that the attainment and maintainance of these levels in man for a long period was not practical. (Fig. 6).

The previous experiment was therefore repeated using 20 mg/kg. All the animals treated in this way died, despite a relatively high level of P2S in the plasma (Fig. 5). This result was unexpected and in sharp contrast to previous results attained by Davies and Willey (4) who showed that 30 mg/kg P2S plus 17.4 mg/kg atropine given intramuscularly, ten minutes before subcutaneous GB, would raise the LD50 in rabbits 30 fold. A careful comparison of the precise conditions under which each set of results were obtained brought to light two essential differences. 1. In the Davies Willey experiments, the agent was given subcutaneously. In the current experiments it was given intramuscularly. 2. In the previous experiments oxime and atropine were given together intramuscularly 10 minutes before the agent: in these experiments oxime was given i.m. 10 minutes before the agent but intramuscular injection of atropine was delayed until 1 minute after poisoning.

One explanation for the discrepancy was the possibility that when the agent and atropine were given intramuscularly a delay of

1 minute resulted in an accumulation of acetyl-choline which was too great to be counteracted by the atropine. To test this, atropine was given intravenously 1 minute after intramuscular injection of 5 x LD50 GB. Despite the fact that a smaller dose of oxime was used, 15 mg/kg, all five animals treated in this way survived. The oxime levels varied from 5-12 μg/ml. In another series of experiments the agent and atropine were given intramuscularly, but the atropine was injected 2 minutes before the agent. Three out of four animals survived at oxime levels varying between 4-11 μg/ml.

When the agent was given subcutaneously and atropine intramuscularly a dose of 15 mg/kg of oxime, which produced levels between 5-10 μg/ml saved 8/9 animals.

With this same procedure 10 mg/kg of oxime saved 4/4 animals with plasma levels of 3-8 μg/ml. With 15 mg/kg of oxime, plasma levels of 3-5.5 μg/ml were obtained and all three animals which received 10 LD50 of GB survived.

4. Plasma levels of P2S and Survival of Rabbits given GB by Inhalation.

In this series of experiments rabbits were exposed to GB at different concentrations and for varying periods, the Ct however, remaining constant,at 1000 ± 50 mg min/m^3.

In the first experiment five rabbits were given 10 mg/kg P2S intramuscularly and 10 minutes later exposed to GB for 1 minute. Blood was taken 3-4 minutes before the commencement of the exposure when the oxime levels were between 3.8 and 7.8 μg/ml. (See Fig. 6). Atropine was given intramuscularly at the onset of signs of poisoning i.e. between 40-60 seconds after the commencement of poisoning. All five animals died.

In a second experiment at the same P2S dosage level the concentration of GB was dropped to 330 mg/m^3 but the time of exposure was increased to 3 minutes. Because signs appeared later i.e. 1-2 minutes - the administration of atropine was correspondingly delayed, otherwise all the conditions were as in the previous experiment. Three out of five animals exposed survived, their plasma levels at the commencement of exposure being 3.3, 6.0 and 7.8 μg/ml. Plasma P2S levels in the two which died were 4.8 and 5.8 μg/ml.

When exposure was increased to 5.0 minutes and the concentration decreased to 200 mg/m^3 in a third experiment, four out of eight animals survived but survival could not be related to the concentration of oxime in the plasma. (See Fig. 6). Increasing the dose of oxime to 15 mg/kg produced higher plasma levels, but although some animals died and others survived, this result was not related directly to the observed level of P2S in the plasma.

This failure to relate oxime level with survival was unexpected, but a possible explanation may be inferred from an inspection of the curves showing how the rise and fall of oxime levels varied between individual animals. This level showed variation in both magnitude and rate of rise and fall, particularly when the dose of oxime was low e.g. 10 mg/kg.

Thus 8 minutes after the intramuscular administration of oxime, the plasma levels in three animals were most probably 3.8, 4.0 and 7.6 μg/ml. (Figure 2). Five minutes later the corresponding figures were 0.6, 2.0 and 4.5 μg/ml respectively. Since a delay of 3-6 minutes, or even longer in some cases, occurred

between blood sampling and commencement of GB exposure it is clear that the recorded oxime levels were higher than those which pertained whilst the animals were being poisoned when the levels were falling.

Finally a definitive experiment was planned following preliminary experiments upon the variation of plasma levels after oral administration of oxime. Given by this route it was shown that the plasma levels varied very little for 1-2 hours after attaining the maximum level (See Figure 4). Variation in individual animals was however still marked.

The oxime was given in delayed release tablet form. Each tablet contained 50 mg P2S. The dose was 100 mg/kg and was given to the nearest tablet or 50 mg.

Blood samples were taken 2 minutes prior to poisoning and also at the end of the 1 minute exposure period. Fifteen animals were exposed and six died. The plasma levels of the six dead animals were 2.0, 3.0, 3.5, 4.1, 5.0 and 6.0 μg/ml. The plasma levels of the survivors lay between 3.9 and 8.9 μg/ml (Figure 7). From this it is seen that if the plasma level is above 4.0 μg/ml the chances of survival are high, but below 4.0 μg/ml they are poor.

5. Plasma levels of P2S and Survival after Oxime and Subcutaneous GB or VX.

100 mg/kg of P2S in delayed release tablet form, were given orally at varying times before the subcutaneous injection of 10 x LD50 GB. Atropine was given intramuscularly either 1.0 minute after poisoning or at the onset of toxic signs. The details of each experiment are shown in Figure 8. In all, observations were made on 31 animals. Fifteen had plasma levels of 4 μg/ml or more of P2S and 13 survived. Sixteen had plasma levels of less than

Footnote. Delayed release tablets were tablets of P2S especially coated to control the release of the oxime with the intestinal tract. They were prepared for human administration but because of their suitability were used in these experiments.

4 μg/ml and 14 died.

A similar series of experiments were carried out with VX. 31 animals were involved. Irrespective of when the atropine was given (See Figure 8), provided the plasma oxime level was 4 μg/ml or greater, the chances of survival were very high, in fact 25 out of 27 survived. If on the other hand it was less than 4 μg/ml, the probability of death was great, 3 out of 4 animals with this level of oxime dying.

6. P2S and Atropine in the Treatment of Dogs poisoned with 10 x LD50 of GB.

Nine dogs were given 15 mg/kg intramuscularly 60 minutes before a subcutaneous injection of 10 x LD50 of GB. Atropine (5 mg/kg) was given 1 minute after the GB. Blood was taken, immediately prior to injection of P2S and also just before the injection of GB. All dogs survived and the plasma levels of P2S at the time of poisoning were between 2.7 and 9.4 μg/ml. In eight of the dogs, the P2S level was between 4.2 and 9.4. Five of them had plasma levels of between 4.2 and 5.7 μg/ml.

DISCUSSION

The present experiments were designed to determine the plasma level of P2S which, when supported by adjuvant atropine (17.4 mg/kg intramuscularly) would save rabbits or dogs from 10 x LD50 of GB or VX administered by inhalation or by parenteral injection. Rabbits and dogs were chosen largely for their experimental convenience e.g. ease of venesection and ready availability.

The results show that with a plasma P2S level of 4 μg/ml or more the chances of survival are high. Statistical evaluation of the data gives a very high significant difference between the survivors with the two levels of oxime concentrations i.e. 4 μg/ml or more and less than 4 μg/ml. When the concentration of the

oxime in the plasma is 4 μg/ml or more, most probably 11% of animals will die but not more than 22% would be expected to die unless there was a 1 in 20 mischance in sampling. On the other hand when the concentration is less than 4 μg/ml 94% will probably die and not less than 76% unless a 1 in 20 mischance in a sampling has come off.

It has long been established that in anticholinesterase poisoning atropine must be given in large doses as soon as possible after indications of poisoning have occurred and also by the fastest possible route. The early experiments here described confirm this. In general the sooner atropine was given after poisoning, the more effective was the treatment; however a number of experiments indicated that atropine administration could still be effective when delayed until the first appearance of objective signs. Delay in giving atropine until the onset of convulsions inevitably caused failure of the treatment.

The critical level in the two species of animals used in the experiments described may not be applicable to primates and man, but allied to other information (1, 4) the goal of 4 μg/ml does not seem to be unreal in the face of a challenge of up to 10 x LD50 doses of GB or VX. The animals were given atropine at the arbitrary times of 1 and 2 minutes after poisoning or on the onset of toxic signs. Men on the other hand could be trained to inject themselves with atropine on the appearance of symptoms, which can be expected to occur earlier than signs, and this would provide a correspondingly increased chance of a successful outcome to the treatment.

However, if man approximates more to dogs than to rabbits the critical P2S plasma level in the face of a 10 x LD50 challenge with GB or VX may be less than 4 μg/ml and levels below this figure can be expected to be effective against a smaller challenge.

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(Sgd.) F.W. Beswick,
Superintendent,
Medical Research Division.

(Sgd.) W.S.S. Ladell,
Assistant Director,
Medical.

DRD/RPB/JCC

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FIG.1.

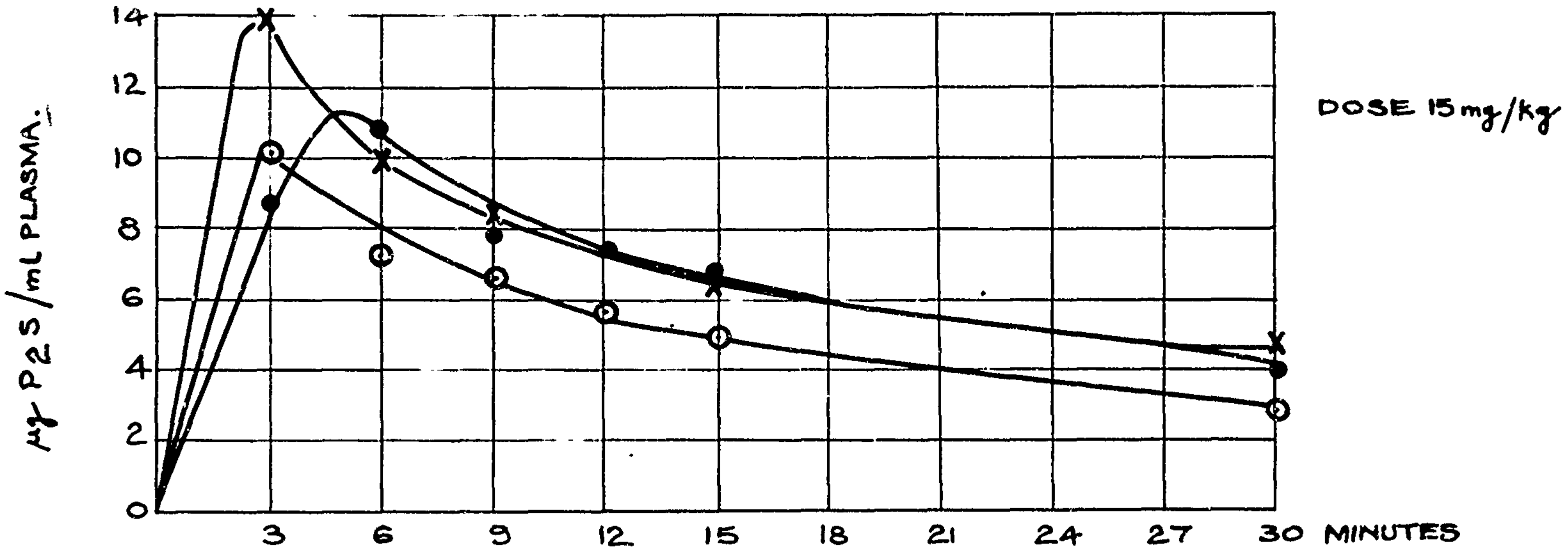

FIG.2.

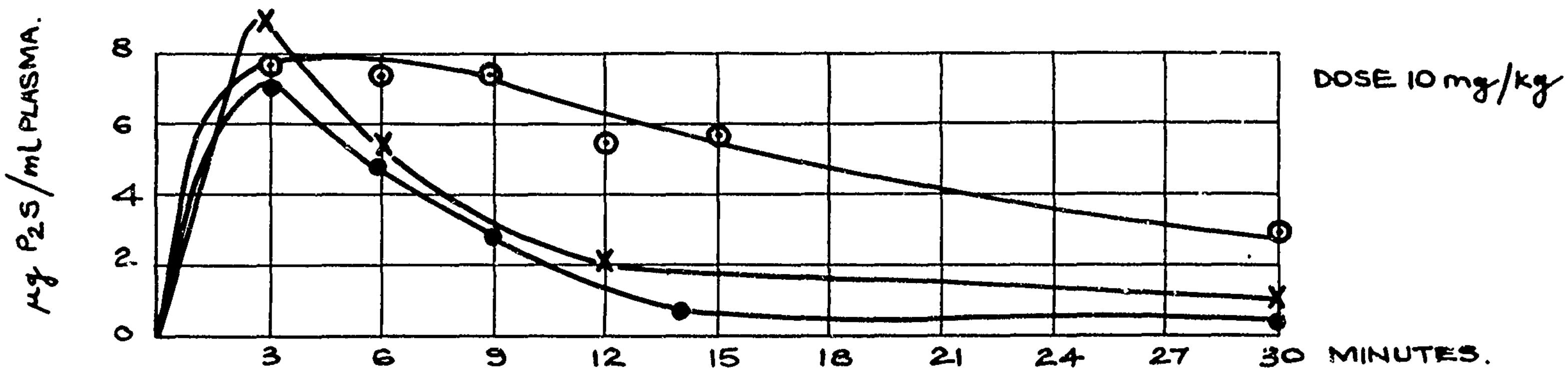

FIG.1.& 2

VARIATION OF P_2S IN PLASMA OF RABBITS AFTER INTRAMUSCULAR ADMINISTRATION.

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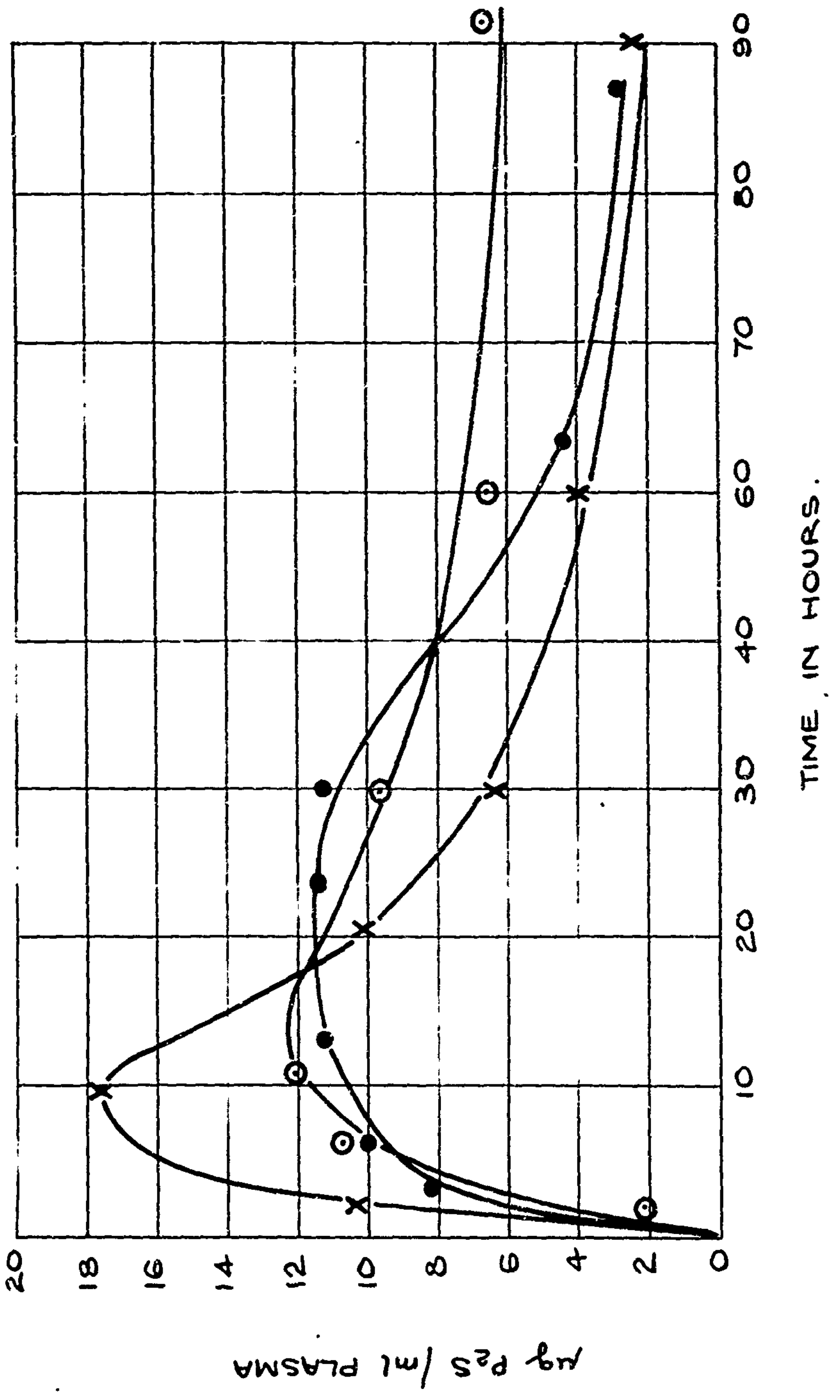

THE VARIATION OF P_2S IN THE PLASMA OF DOGS.

FOLLOWING I.M. ADMINISTRATION OF 15 mg/kg.

FIG. 3.

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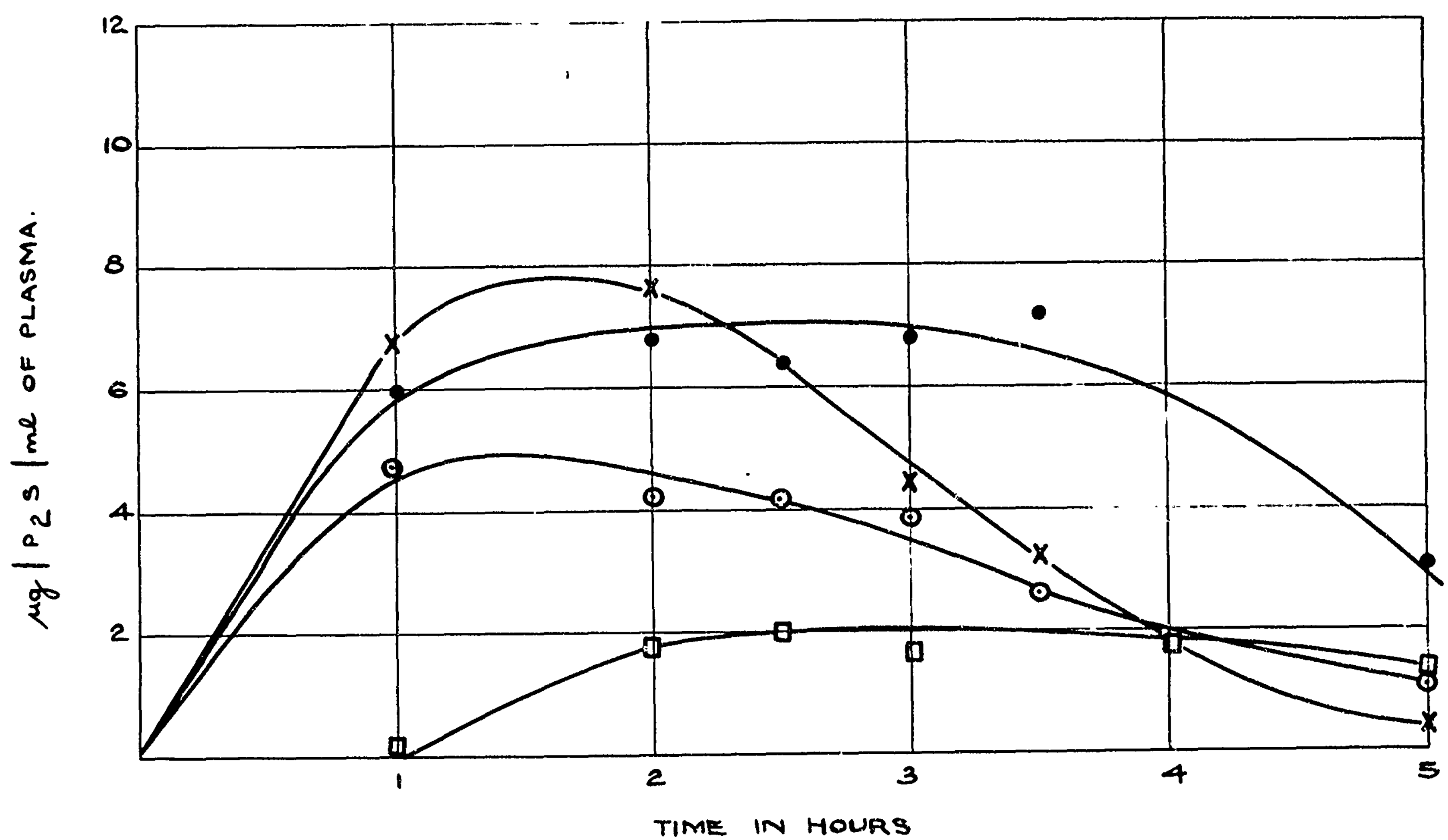

THE VARIATION OF P_2S IN THE PLASMA OF RABBITS FOLLOWING ORAL ADMINISTRATION OF 100 mg/kg.

FIG. 4.

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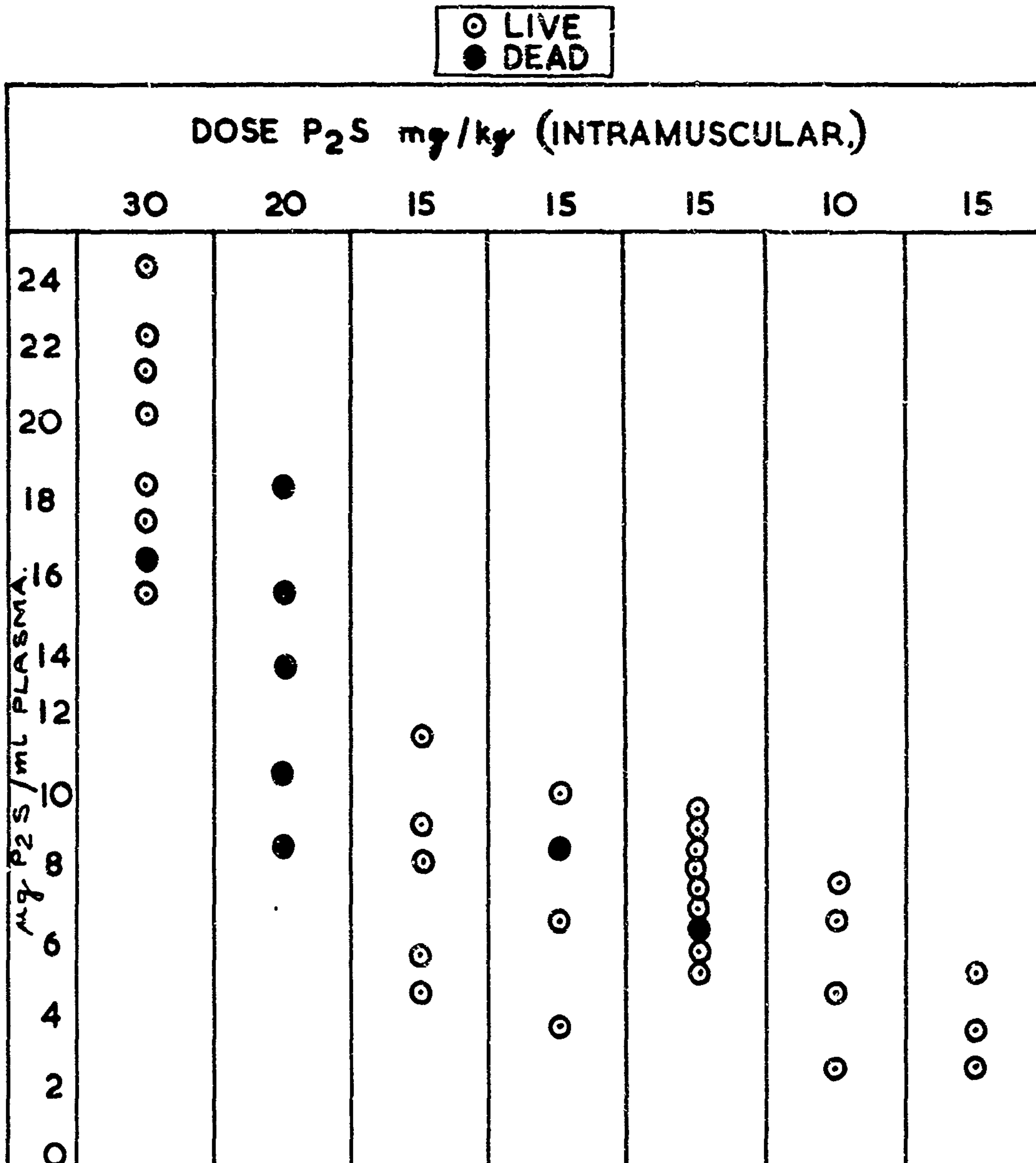

DOSE GB	$5 \times LD_{50}$	$5 \times LD_{50}$	$5 \times LD_{50}$	$5 \times LD_{50}$	$5 \times LD_{50}$	$5 \times LD_{50}$	$10 \times LD_{50}$
ROUTE GB	I.M.	I.M.	I.M.	I.M.	SUB-CUT	SUB-CUT	SUB-CUT
ROUTE Atrop	I.M.	I.M.	I.V.	I.M. 2 MINS BEFORE GB	I.M.	I.M.	I.M.

THE EFFECT OF ROUTES OF ADMINISTRATION OF GB AND ATROPINE UPON THE SURVIVAL OF RABBITS GIVEN P_2S INTRAMUSCULARLY.

FIG.5.

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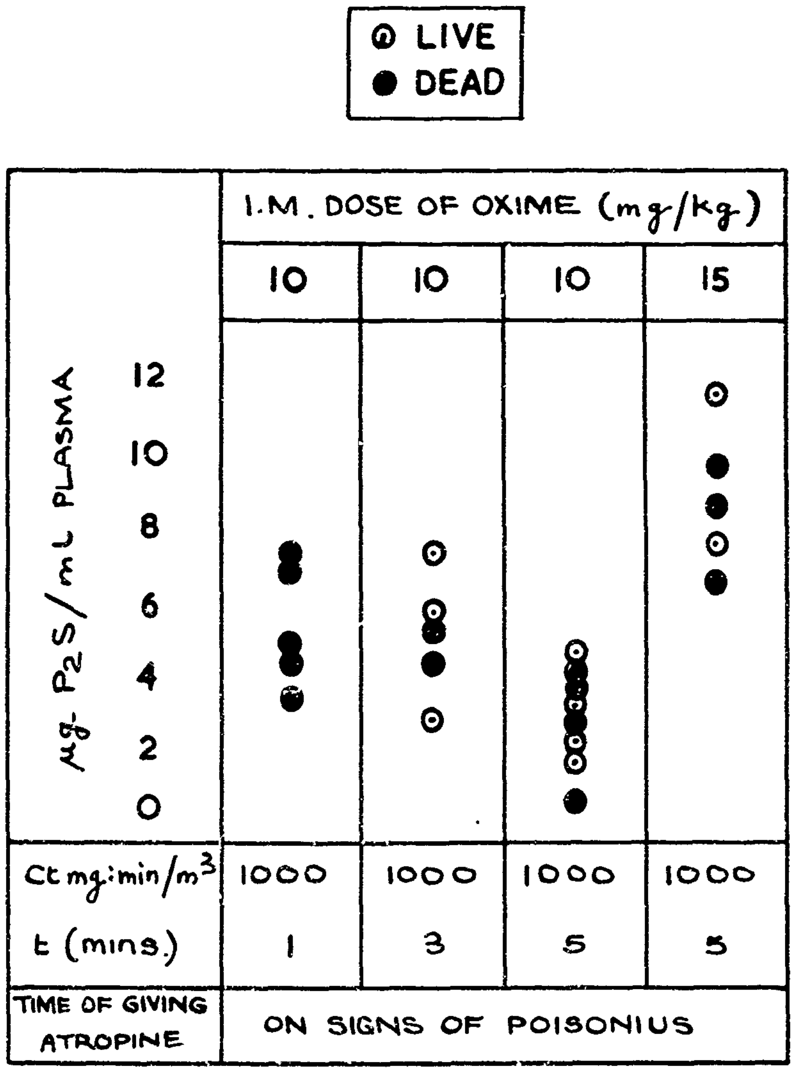

PLASMA LEVELS OF P_2S AND SURVIVAL OF RABBITS INHALING 10 X LCt_{50} OF GB INFLUENCE OF DOSE AND TIME OF ADMINISTRATION OF P_2S.

FIG. 6.

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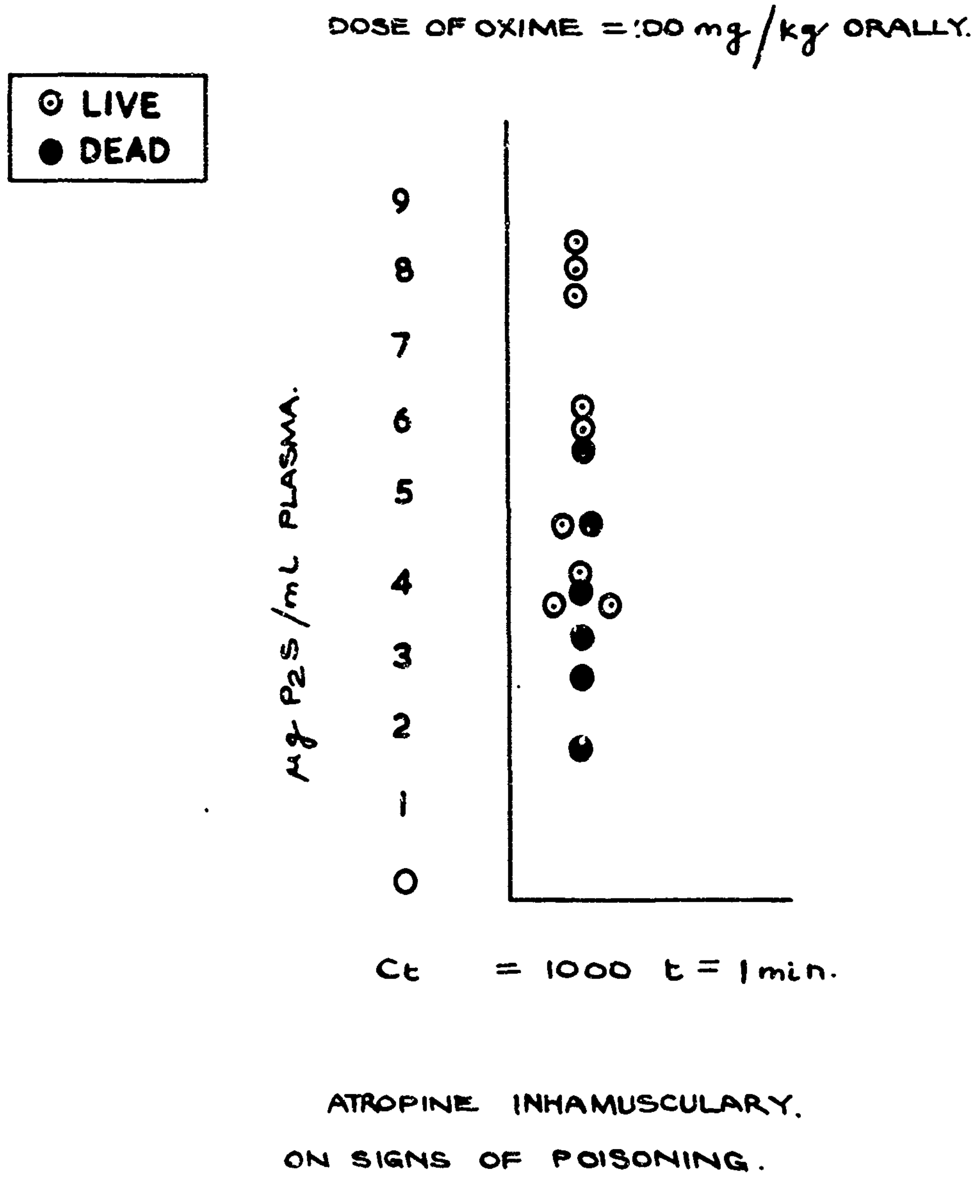

PLASMA LEVELS OF P_2S AND SURVIVAL OF RABBITS GIVEN GB BY INHALATION AND OXIME ORALLY.

FIG.7.

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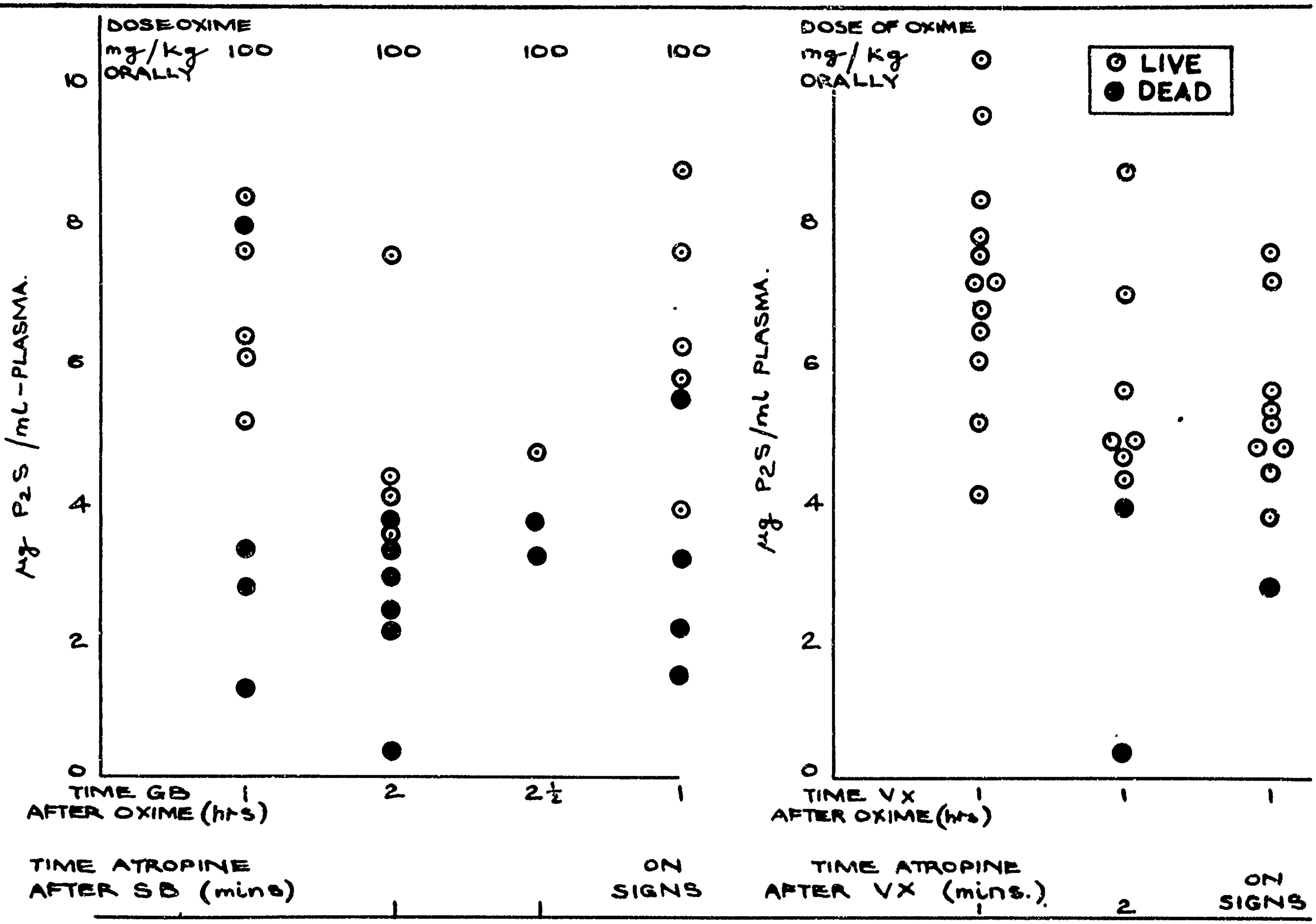

PLASMA LEVELS OF P_2S AND SURVIVAL AFTER POISONING WITH

10 x LD_{50} OF GB OR VX SUBCUTANEOUSLY.

FIG.8.

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